

***Pseudobaeospora lilacina* sp. nov., the first report of the genus from China**

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ABSTRACT—A new species, *Pseudobaeospora lilacina*, is described based on materials collected from Guangdong Province, China. The fungus represents the first record of the genus in China. The new species is characterized by its lilac pileus, stubbly stipe, and ellipsoid spores. Phylogenetic analyses of the internal transcribed spacer regions (ITS) and nuclear ribosomal RNA gene large subunit (nrLSU) support *P. lilacina* within *Pseudobaeospora* and as distinct from all other sequenced species of the genus. The combination of morphological and molecular data confirmed the fungus to be new. The new species is compared to morphologically similar species and its taxonomic position is discussed.

KEY WORDS —Agaricales, Basidiomycota, taxonomy, Tricholomataceae

Introduction

Pseudobaeospora Singer was erected by Singer (1942) and emended by Bas (2003); the genus is characterized by small basidiocarps, pale to dark lilac pilei, and small spores. Bas (2003), who described 25 *Pseudobaeospora* species, divided the genus into five groups (Albidula, Celluloderma, Pyrifer, Frieslandica, and Pillodii) based primarily on basidiocarp color and the presence or absence of clamp connections. Kirk et al. (2008) estimated about 20 species for the genus, while the current Index Fungorum database (<http://www.indexfungorum.org> accessed June 2016) lists 34 published taxa.

The type species of the genus, *Pseudobaeospora oligophylla* (Singer) Singer, was originally found in the Altai Mountains of central Asia by Singer (1938; as *Baeospora oligophylla*). *Pseudobaeospora* species were subsequently described from Asia, Europe, and North and South America (Singer 1986). Additional species have been described from California (Vellinga 2001, 2009; Schwarz 2012), Hawaii (Desjardin et al. 2014), and Slovakia (Adamčík & Jančovičová 2011), but the genus remained unreported from China.

The taxonomic position of *Pseudobaeospora* has been ambiguous. Singer (1942) initially placed the genus in *Tricholomataceae* as an independent genus but soon afterwards transferred it to *Agaricaceae*, at first in tribus *Lepioteae* (Singer 1951) and later in tribus *Cystodermateae* (Singer 1986). However, Kühner (1980) retained the genus in *Tricholomataceae*, a placement supported by subsequent researchers (Bas 2003, Desjardin et al. 2014).

Here we describe a new species of *Pseudobaeospora* from China based on a combination of morphological and molecular data; we also compare the morphological similarities and differences between the new *P. lilacina* and previously described *Pseudobaeospora* species.

Material & methods

Fungal collections

Fungal specimens were collected from Guangdong Province, China. Tissue blocks were removed from the inner part of fresh basidiomata for DNA analyses. The specimens were dried with an electric air-ventilation drier and deposited in either the Fungal Herbarium of Shenyang Agricultural University, Shenyang, China (SYAU), or the Mycological Herbarium of Guangdong Institute of Microbiology, Guangzhou, China (GDGM).

Morphological observations

The macroscopic description is based on the fresh specimens. The names of colors are based on Ridgway (1912). Microscopic observations were made on sections of dried specimens, using a 5% KOH solution and in Melzer's reagent, using a light microscope. The Q-value (quotient of length/width) for each spore (20 spores for each specimen) was calculated, and the mean values were used in the descriptions.

For observation of the surface of spores, the gills were coated with gold. The spores were examined with a scanning electron microscope (SEM; Zeiss & Ultra Plus, Germany).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the fresh blocks of tissue of the specimens using the Plant DNA Extraction Kit (Sunbiotech Co., Ltd, Beijing). The raw DNA extracts were used as templates for PCR. Amplification primers were ITS5/ITS4 (White et al.

1990) for the ITS1+5.8S+ITS2 region and LROR/LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>) for the 5' end of 28S rRNA gene. Both reaction mixtures and PCR conditions followed those in Yu et al. (2014). The PCR products were checked on a 1% agarose gel stained with ethidium bromide. The DNA bands were visualized on a UV transilluminator and documented digitally using the BIO-RAD ChemiDoc XRS imaging system. Sequencing was performed on an ABI Prism® 3730 Genetic Analyzer (PE Applied Biosystems, USA).

DNA sequence analyses

The BLAST database (Altschul et al. 1997) was searched using the ITS and nrLSU sequences from the type collection (SYAU-FUNGI-009) as the queries (23 May 2016). In order to confirm the taxonomic position of the new species, the sequences of the Tricholomatoid clade (Matheny et al. 2006) were also retrieved from GenBank. Nucleotide sequences amplified from the collections of the new species were aligned with 44 ITS and 51 LSU sequences retrieved from GenBank using BioEdit 5.0.6 (Hall 1999) and Clustal X (Thompson et al. 1997). Based on the results of Matheny et al. (2006), the sequences of *Agaricus bisporus* (J.E. Lange) Imbach (DQ404388 for ITS; AY635775 for nrLSU) were downloaded and included for rooting purposes.

A few dozen bases at both ends of the sequences were excluded from the analyses because of the uncertainty in the base calls of the sequences. Finally, the data matrices with 689 bp for ITS and 955 bp for nrLSU were produced. Nodal bootstrap support (BS) was assessed with nonparametric bootstrapping using 1000 replicates. Bayesian analysis was conducted using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). The best-fitting sequence evolution model was chosen using MrModelTest v. 2.2 (Nylander 2004). Bayesian analyses of the ITS and nrLSU regions were run for 2 000 000 generations, all under the GTR model, with four chains, and trees sampled every 100 generations. The average split frequencies were checked to determine optimal convergence of the chains below 0.01 after each generation. The first 25% (1250 trees) of the sample trees was designated as burn-in, with the remaining samples retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree for Posterior Probabilities (PP). Alignments have been deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S19825> and S19826).

Results

Four ITS sequences and four nrLSU sequences were obtained from fresh basidiomata. The average standard deviation of split frequencies in the Bayesian analysis was less than 0.01 after 2,000,000 generations. Both trees were rooted with *Agaricus bisporus* (FIGS. 1, 2). The ITS and nrLSU sequence analyses both placed the genus *Pseudobaeospora* in a monophyletic group (PP = 1) sister to *Callistosporium graminicolor* Lennox (*Tricholomataceae*). The new species, *P. lilacina*, grouped with the other *Pseudobaeospora* species (*P. deckeri* C.F. Schwarz, *P. pyrifer* Bas & L.G. Krieglst., *P. wipapatiae* Desjardin et al.) but formed a separate branch with strong support (PP = 1).

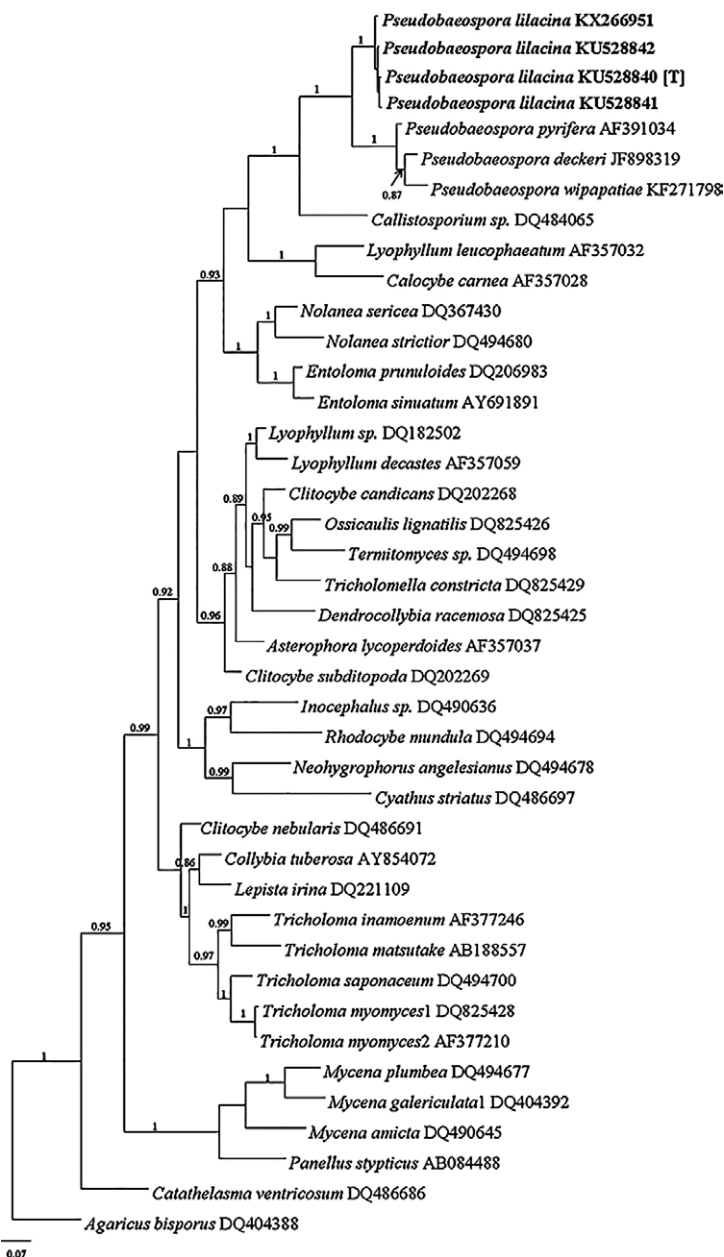


FIG. 1. Fifty percent majority-rule Bayesian cladogram of *Pseudobaespora* and related taxa based on ITS sequence analysis. Node support is indicated by Bayesian posterior probabilities >0.5 on branches. *Pseudobaespora lilacina* sequences are presented in boldface.

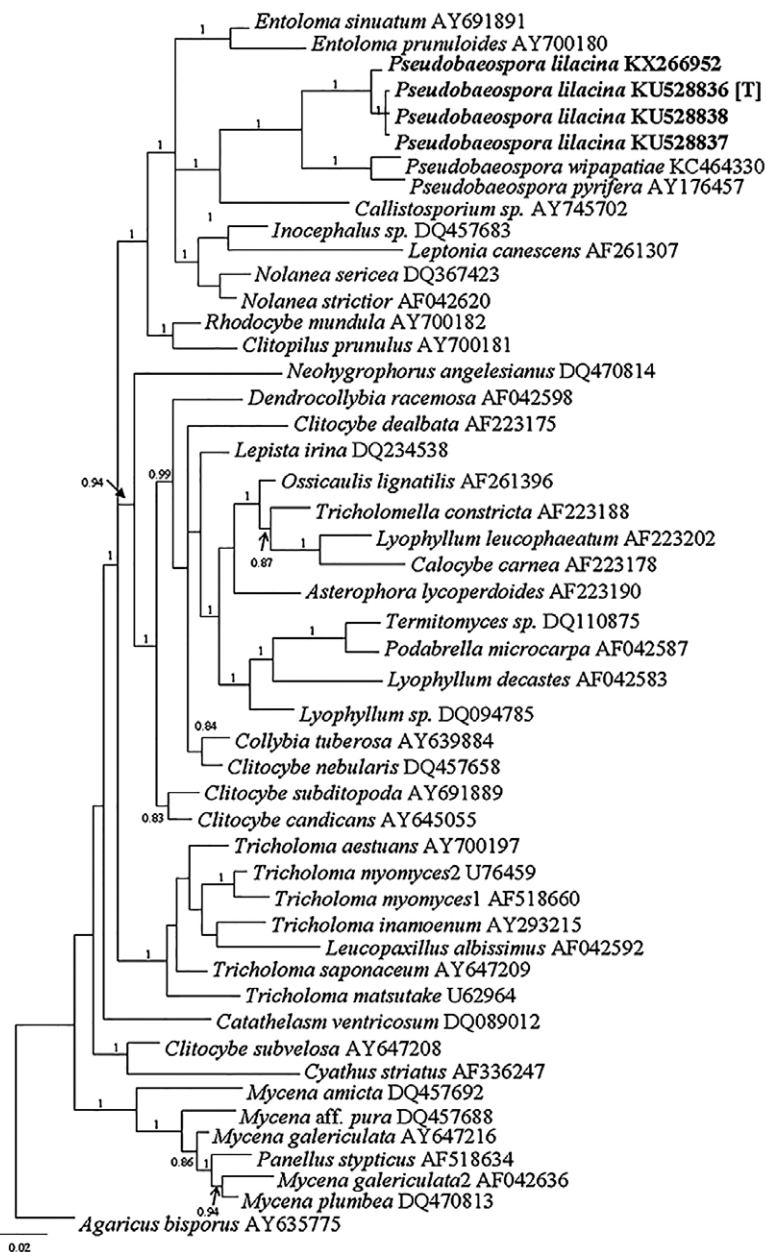


FIG. 2. Fifty percent majority-rule Bayesian cladogram of *Pseudobaeospora* and related taxa based on nrLSU sequence analysis. Node support is indicated by Bayesian posterior probabilities >0.5 on branches. *Pseudobaeospora lilacina* sequences are presented in boldface.

Taxonomy

Pseudobaeospora lilacina X.D. Yu, Ming Zhang & S.Y. Wu, **sp. nov.**

FIG. 3

MYCOBANK MB 815567

Differs from other species in the *Frieslandica* group by its lilac pileus, stubbly stipe, and ellipsoid spores.

TYPE: China. Guangdong Province, Guangzhou City, Baiyun Mountain, on grass, 28 May 2013, Zhang M 3459 (**Holotype**, SYAU FUNGI-009; GenBank KU528840, KU528836).

ETYMOLOGY: The epithet *lilacina* refers to the lilac pileus color.

PILEUS 1–3 cm diam, plano-convex to almost flat, dry; surface pale mauve, colonial buff when old, slightly squamulose, darker at center, hygrophanous; margin flexuous to involute. CONTEXT 0.1–0.2 cm thick at stipe, thin at margin, whitish to tinged with pileus' color. LAMELLAE adnate to uncinata, arched, ≤ 3 mm broad, unequal, distant, with lamellulae of 2–4 lengths but not anastomosing, pale mauve to mauve, undulate and thin at edge. STIPE 2–3 $\times$ 0.2–0.5 cm, cylindrical, somewhat expanded at base, sometimes pliant, pale Congo pink, fibrous, striate, central, pruinose to flocculose overall, bruising indigo-bluish. ODOR inconspicuous.

BASIDIOSPORES 2.5–3.5(–4.5) $\times$ 3–5(–6.5) μm , $Q = 1.38$, broadly ellipsoid to ellipsoid, hyaline, thick-walled, dextrinoid, minutely rough and with a distinct hilar appendage under scanning electron microscope. BASIDIA 14–25 $\times$ 3.0–5.8 μm , 4-spored and 2-spored. HYMENIUM hyphae 3.5–7 μm diam, hyaline, thin-walled, non-amyloid; cystidia absent. HYMENOPHORAL TRAMA 100–180 μm wide, regular, hyphae 2.8–7.3 μm , thin-walled. PILEIPELLIS a trichoderm of ascending cylindrical hyphae, 6–10 μm diam, thin-walled, hyaline; suprapellis composed of regular parallel hyphae, 4.2–8.3 μm diam, thin-walled, hyaline, color unchanging in 5% KOH. CLAMP CONNECTIONS present in all parts of the basidiocarp.

HABIT saprophytic, solitary on naked soil or on grass.

ADDITIONAL SPECIMENS EXAMINED: CHINA. GUANGDONG PROVINCE, Zhaoqing City, Fengkai County, Heishiding Nature Reserve, 3 June 2013, Yu XD 5546 (SYAU FUNGI-011; GenBank KU528842, KU528838); Guangzhou City, Baiyun Mountain, 28 May 2013, Yu XD 3450 (SYAU FUNGI-010; GenBank KU528841, KU528837); 22 June 2008, Li TH, Chen XL, Li Y (GDGM25609; GenBank KX266951, KX266952); 22 May 2011, Xu J (GDGM44799); 25 May 2011, Xu J, Chen XL, Zhang M (GDGM28870); 27 May 2011, Xu J, Qiu CS, Zhang M (GDGM28919).

Discussion

Pseudobaeospora lilacina represents the first report of *Pseudobaeospora* in China. Based on morphology, the new species should be placed in the

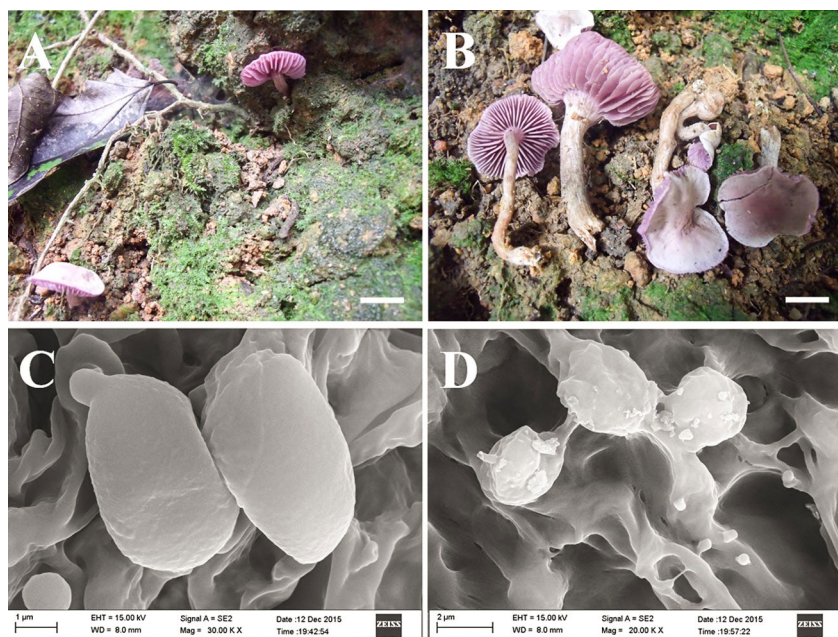


FIG. 3. *Pseudobaeospora lilacina* (holotype, SYAU FUNGI-009). A, B: Macroscopic habit. C, D: Surface of spores. Scale bars: A, B = 1 cm; C = 1 μ m; D = 2 μ m.

Frieslandica group: colored basidiocarp, presence of clamp connections, and non-hymenidermoid pileipellis (Bas 2003). However, its pale mauve pileus and stubbly stipe easily distinguish *P. lilacina* from the other Frieslandica species. Although most of the other species have purple tones in the pileus, their colors are conspicuously deeper (Bas 2003) than those found in *P. lilacina*. *Pseudobaeospora subglobispora* Bas from Asia shares a similar pileus color, but is distinguished from *P. lilacina* by its shorter (0.4–1 cm) stipe and obvious cheilocystidia. Three additional Asian species—*P. citrina* Rawla, *P. lavendulamellata* Arnolds et al., *P. oligophylla*—are easily distinguished from *P. lilacina*: *P. citrina* by a greenish yellow pileus (Bas 2003), *P. lavendulamellata* by a dull violet pileus (Arnolds et al. 2004), and *P. oligophylla* by a dark violaceous pileus (Bas 2003). Furthermore, in *P. oligophylla* there are no clamp connections in the basidiocarp hyphae (Bas 2003), and *P. lavendulamellata* has obvious cheilocystidia and its pileipellis discolors in 5% KOH (Arnolds et al. 2004).

We found only three *Pseudobaeospora* species—*P. deckeri*, *P. pyrifer*, *P. wipapatiae*—with available GenBank sequences, indicating the deficiency of molecular research on the genus. However, our molecular analyses provide some clues as to the taxonomic position of *Pseudobaeospora*. Both ITS and nrLSU sequence analyses (FIGS 1, 2) placed the *Pseudobaeospora* species and *Callistosporium graminicolor* into one clade supported by strong Posterior Probabilities (PP = 1), with the clade clustering with *Lyophyllaceae* or *Entolomataceae*. Our result is inconsistent with the previous nrLSU sequence analysis by Desjardin et al. (2014), which placed *Pseudobaeospora* in *Tricholomataceae*. The reason for the inconsistency is not clear but may be explained by the low number of sequences available or taxa selected for analysis. Even so, our result supports the observation by Vellinga (2004) that *Pseudobaeospora* does not belong in *Agaricaceae*.

Pseudobaeospora will require more sampling and molecular analyses before its taxonomic position can be established.

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